ehp ENVIRONMENTAL HEALTH PERSPECTIVES
http://www.ehponline.org

Transdermal Uptake of Diethyl Phthalate and Di(*n*-butyl) Phthalate Directly from Air: Experimental Verification

Charles J. Weschler, Gabriel Bekö, Holger M. Koch, Tunga Salthammer, Tobias Schripp, Jørn Toftum, and Geo Clausen

http://dx.doi.org/10.1289/ehp.1409151

Received: 30 August 2014
Accepted: 3 April 2015
Advance Publication: 7 April 2015

Transdermal Uptake of Diethyl Phthalate and Di(*n*-butyl) Phthalate Directly from Air: Experimental Verification

Charles J. Weschler,[1,2] Gabriel Bekö,[2] Holger M. Koch,[3] Tunga Salthammer,[4] Tobias Schripp,[4] Jørn Toftum,[2] and Geo Clausen[2]

[1]Environmental and Occupational Health Sciences Institute, Rutgers University, Piscataway, New Jersey, USA; [2]International Centre for Indoor Environment and Energy, Department of Civil Engineering, Technical University of Denmark, Lyngby, Denmark; [3]Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, Institute of the Ruhr-Universität Bochum (IPA), Bochum, Germany; [4]Fraunhofer WKI, Department of Material Analysis and Indoor Chemistry, Braunschweig, Germany

Address correspondence to Charles J. Weschler, Environmental and Occupational Health Sciences Institute, Rutgers University, 170 Frelinghuysen Road, Piscataway, NJ 08854 USA. Telephone: 848-445-2073. E-mail: weschlch@rwjms.rutgers.edu

Running title: Evidence for dermal uptake of phthalates from air

Acknowledgments: The authors are grateful to Nico Ziersen and Louise B. Weschler for their assistance with these experiments, to the participants for their time and care following the restrictions that were part of this study, and to Glenn Morrison for useful comments and suggestions throughout.

Competing financial interests: All authors declare no actual or potential competing financial interests.

Abstract

Background: Fundamental considerations indicate that, for certain phthalate esters, dermal absorption from air is an uptake pathway that is comparable to or larger than inhalation. Yet this pathway has not been experimentally evaluated and has been largely overlooked when assessing uptake of phthalate esters.

Objectives: This study investigated transdermal uptake, directly from air, of diethyl phthalate (DEP) and di(*n*-butyl) phthalate (DnBP) in humans.

Methods: In a series of experiments, six human participants were exposed for six hours in a chamber containing deliberately elevated air concentrations of DEP and DnBP. The participants either wore a hood and breathed air with phthalate concentrations substantially below those in the chamber or did not wear a hood and breathed chamber air. All urinations were collected from initiation of exposure until 54 hours later. Metabolites of DEP and DnBP were measured in these samples and extrapolated to parent phthalate intakes, corrected for background and hood air exposures.

Results: For DEP the median dermal uptake directly from air was 4.0 μg/(μg/m^3 in air) compared with an inhalation intake of 3.8 μg/(μg/m^3 in air). For DnBP the median dermal uptake from air was 3.1 μg/(μg/m^3 in air) compared with an inhalation intake of 3.9 μg/(μg/m^3 in air).

Conclusions: This study shows that dermal uptake directly from air can be a meaningful exposure pathway for DEP and DnBP. For other semivolatile organic compounds (SVOCs) whose molecular weight and K_{ow} are in the appropriate range, direct absorption from air is also anticipated to be significant.

Introduction

Selected phthalates have been associated with multiple health effects, including children's neurodevelopment (Kim Y et al. 2011; Whyatt et al. 2012), altered genital development (Swan et al. 2005), respiratory problems (Jaakkola and Knight 2008), oxidative stress (Ferguson et al. 2011), obesity (Hatch et al. 2010), and the development of diabetes (Svensson et al. 2011). Phthalates are used in a wide variety of consumer products (Dodson et al. 2012), and their metabolites are commonly found in human urine (Koch et al. 2011; Langer et al. 2014; Zota et al. 2014). Several studies have assessed the relative contribution of diet, dust ingestion, inhalation or dermal absorption to the total level of selected phthalates in humans (Bekö et al. 2013; Gaspar et al. 2014; Guo and Kannan 2011; Koch et al. 2013; Wittassek et al. 2011; Wormuth et al. 2006). Prior to 2012, assessments of the dermal pathway have focused on contact transfer, including transfer from dust deposited on skin. Kissel (2011) has argued that dermal absorption has been underestimated in many studies that have used an inappropriate metric (percent absorption) to assess dermal uptake. Prompted by his critique, there has been recent interest in dermal absorption of phthalates and other semivolatile organic compounds (SVOCs) directly from air (Weschler and Nazaroff 2012; 2014; Gong et al. 2014a). Numerous experimental studies have demonstrated that certain volatile organic compounds (VOCs) are dermally absorbed from air at rates that are comparable to their intake via inhalation (e.g., Bader et al. 2008; Piotrowski 1967; 1971; other references in Weschler and Nazaroff, 2014). Although fundamental physical-chemical considerations indicate that the same should be true for certain semivolatile organic compounds (SVOCs), we are aware of no investigations that have measured direct dermal uptake from air for any SVOC.

Diethyl phthalate (DEP) and di(n-butyl) phthalate (DnBP) are among the SVOCs predicted to have substantial dermal uptake directly from air (Weschler and Nazaroff 2012; 2014). DEP and DnBP belong to the group of so-called low molecular weight phthalates with two and four carbon atoms in the alkyl chain, respectively. Their chemical and physical properties are summarized in Supplemental Material, Table S1. DEP is used in personal care products such as cosmetics, perfumes and shampoos; it is also used in the automotive sector and food packaging; DnBP is a common ingredient of adhesives and coatings and used as a solvent for organic compounds, as an antifoam agent, as a fiber lubricant and as an additive in cosmetics including nail polish (Bolgar et al. 2008; Dodson et al. 2012; Wypych, 2012). DEP and DnBP have been identified in indoor air and dust samples from around the world (e.g., Fromme et al. 2004; Rudel et al. 2003; Wensing et al. 2005; Weschler and Nazaroff 2008). Their metabolites are often the most abundant synthetic chemicals identified in human urine (Koch et al. 2011; Langer et al. 2014; Zota et al. 2014). The aim of the present study has been to experimentally evaluate transdermal uptake of DEP and DnBP directly from the gas phase based on their metabolites in urine. This was accomplished by differential inclusion and exclusion of the inhalation pathway during repeated controlled exposures of human participants in a large chamber.

Methods

Human participants

Six healthy males, 27 to 66 years old, agreed to participate in these experiments. Their physiological parameters are summarized in Supplemental Material, Table S2. None had an identified skin disease or skin that was compromised by a wound. The research protocol was

approved by the Capital Region of Denmark Committee for Research Ethics (case no. H-3-2013-196). Participants provided informed consent before participation.

Exposure experiments

The six participants were divided into Groups 1 and 2, each with three subjects. The exposures occurred from 10:00 to 16:00 on Tuesdays and Wednesdays of successive weeks and followed a crossover design. Group 1 was exposed on Tuesdays, with hoods the first week and without hoods the second week. Group 2 was exposed on Wednesdays, without hoods the first week and with hoods the second week. The participants wore only shorts during their six-hour exposures. They began their restricted diet and restricted use of personal care products (see below) 12 hours before entering the chamber and continued these restrictions until the end of urine collection 66 hours later.

Restricted diet and restricted use of personal care products

To better distinguish differences in the concentrations of DEP and DnBP metabolites in urine resulting from the chamber exposure, the participants followed a restricted diet and avoided all personal care products from 12 hours before until 54 hours after exposure began. While on the restricted diet, participants were only permitted tap water, tea brewed with tap water, Swedish dried bread and fruits with thick skins (e.g., bananas, oranges, melons). Avoidance of personal care products included no use of soaps, shampoos, deodorants, colognes or toothpaste. Preliminary experiments were conducted with eight participants over a 48 hour period to evaluate the efficacy of these restrictions. Background urine concentrations were reduced to medians/means and ranges of 4.1/11.8 and 0.9-53 μg/L for MEP and 7.4/10.7 and 1.5-37 μg/L for MnBP. The background levels achieved with restrictions were below median background

levels in the general population (30 µg/L for MEP and 9.2 µg/L for MnBP for adult males sampled in 2011/2012 (CDC 2015)), and more than two orders of magnitude below peak levels observed in the subsequent exposure experiments.

Breathing hoods and breathing air

Supplemental Material, Figure S1 shows one of the authors wearing a breathing hood. The breathing hoods and associated latex neck seals and hoses were purchased from Amron International, Vista, CA (#8890 Oxygen Treatment Hood). Samples cut from each were ultrasonically extracted with acetone and analyzed by GC/MS for both DEP and DnBP using a DB-5MS column and SIM (m/z 149); their levels were found to be negligible. The level of another phthalate plasticizer, di(2-ethylhexyl) phthalate (DEHP), was quite high. However, the metabolites of DEHP differ from those of DEP and DnBP (Koch et al. 2013), and do not interfere with the analyses of MEP, MnBP and 3OH-MnBP. The breathing air was compressed air that passed through both particulate and activated carbon filters before flowing through the hood at 50 liters/minute. It was not humidified. Between the first and second round of experiments, duplicate one hour air samples were collected from one of the breathing hoods under conditions similar to when the participants were wearing hoods. The measured levels of DEP in the hood with breathing air flowing through were 42.5 and 39.0 µg/m^3 while those of DnBP were 6.3 and 5.0 µg/m^3. Since negligible amounts of these phthalates had been measured in the hoods, neck seals and hoses, we assume that the source of DEP and DnBP was upstream of the hoods. Based on these measurements, all doses reported during the period that the participants were wearing hoods were corrected for inhalation of DEP and DnBP in hood air

assuming a breathing rate of 0.7 m^3/h (US EPA 2011). Further details are provided in subsection "*Background and hood air corrections; normalization*".

Preparing the chamber for the exposure experiments

The exposure experiments were conducted at the Technical University of Denmark (DTU) in a sparsely furnished 55 m^3 chamber whose air was mixed by two fans. Only one group at a time was exposed in the chamber. Each participant sat on a wire mesh chair at a small table and typically worked on their laptop while in the chamber. Supplemental Material, Figure S2 shows Group 1 participants in the chamber wearing breathing hoods. The air exchange rate was maintained at 0.7 /h, while the temperature was controlled at 30 ^oC. The relative humidity was not controlled and ranged between 20 and 35% when the participants were in the chamber. Chamber conditions for the experiments are summarized in the Supplemental Material, Table S3.

Latex paint, formulated with 1% DEP and 10% DnBP (by weight), was used to deliver these phthalates into chamber air at a relatively constant emission rate. Further details regarding the paint, including experiments conducted to determine the mass fraction of DEP and DnBP required to achieve the targeted steady-state air concentrations, are described in Schripp et al. 2014.

On Monday of the first exposure week, the latex paint spiked with DEP and DnBP was applied on each side of 6 aluminum plates (1 m x 1 m; total area of 12 m^2) with a paint roller, and the plates were immediately placed in the chamber. The following Monday the panels were re-painted following the same procedure. Each week, during the 6-hour exposures that occurred on Tuesday and Wednesday, the gas phase concentrations of the two phthalates were determined on an hourly basis. The average gas phase concentrations measured during the four exposure

periods are shown in Table 1, while hourly values measured during the exposure periods are plotted in Supplemental Material, Figure S3. For the exposure periods when hoods were worn, the air concentrations of DEP and DnBP for Group 1 were 12% lower than those for Group 2, while for the exposure periods without hoods, the air concentrations for Group 2 were about 20% lower than those for Group 1 (Table 1).

Measurement of phthalate metabolites in urine

On the mornings of exposure each participant collected one or two urine samples prior to entering the chamber and, after entering the chamber, all urinations for the next 54 hours – recording the time of the void for each individual sample. Urine voids were collected in 250 ml polypropylene specimen containers known to be phthalate-free; during some urinations more than one vessel was used to collect the urine. Field blanks were not collected given the many different microenvironments occupied by the participants after leaving the chamber. However, the target analytes – MEP, MnBP and 3OH-MnBP – are not common contaminants in indoor settings. The samples were weighed the day of collection and then stored in a freezer until they were shipped overnight to the Institute for Prevention and Occupational Medicine of the German Social Accident Insurance (the IPA) in Bochum, Germany. Samples were still frozen upon arrival.

Urine samples were analyzed for mono-ethyl phthalate (MEP), a metabolite of DEP, as well as mono-n-butyl phthalate (MnBP) and 3OH-mono-n-butyl phthalate (3OH-MnBP), metabolites of DnBP. The concentrations of these metabolites were determined by two-dimensional high performance liquid chromatography coupled to tandem mass spectrometry (LC/LC–MS/MS) using internal isotope-labeled standards after enzymatic deconjugation of the phthalate

metabolites from the glucuronidated form following methods published by Koch et al. (2003, 2007) and Preuss et al. (2005). The limits of quantification (LOQ) for MEP, MnBP and 3OH-MnBP were 0.25, 1.0 and 0.25 µg/L, respectively. Quality control materials, prepared using pooled native urine from various individuals from the IPA to represent the general conjugation status (e.g., glucuronidation) of all urine samples, and reagent blank samples were included in each batch together with the study samples. The laboratory that performed the analysis has successfully participated as a reference lab for phthalate metabolite analyses in the quality assessment/quality assurance program of the European Union (EU) financed Consortium to Perform Human Biomonitoring on a European Scale (Schindler et al. 2014). The creatinine concentration in urine was measured according to the Jaffé method (Taussky 1954).

Chemical analysis

Air samples were collected using stainless-steel tubes filled with Tenax TA at 100 mL/min (6 L total sampling volume). The tubes were analyzed via thermal desorption (TD100, Markes Ltd.)/gas chromatography (6890 Series GC System, Agilent)/mass spectrometry (5973N MSD, Agilent) according to DIN ISO 16000-6, 2012. The GC was equipped with a HP5MS column (60 m x 250 µm x 0.3 µm); the oven temperature program was 30°C (1 min) with 8°C/min to 280°C (5 min). The MSD was operated in selected ion monitoring mode (SIM) using m/z 149 (phthalates) and m/z 234 (internal standard). Substances were quantified on the basis of original standards. 3-Bromo-biphenyl was used as an internal standard during sampling and analysis. The detection limit for DEP and DnBP as determined from the calibration curve (Einax et al. 1997) was 1 µg/m³.

Calculating uptakes from metabolite concentrations measured in urine

We have calculated the total uptake from the metabolite levels in urine using established procedures (Koch et al. 2007; 2012; 2013). The molecular weights (g/mol) used to convert the metabolites to the parent compounds are: DEP: 222.24, MEP: 194.18, DnBP: 278.34, MnBP: 222.24 and 3OH-MnBP: 238.24. From metabolism studies (Koch et al. 2012) we have metabolic conversion factors, indicating the fraction of an (oral) dose of parent phthalate that is excreted as a specific metabolite in urine: DEP/ MEP: ~ 0.84 (set by analogy to DnBP); DnBP/MnBP: 0.84; and DnBP/3OH-MnBP: 0.07. DnBP uptake was calculated by summing up both metabolites:

$$\text{DnBP } (\mu g) = [\{(\text{MnBP } /222.24) + (3\text{OH-MnBP } /238.24)\} * 278.34] / (0.84+0.07) \quad [1]$$

DEP uptake was calculated as:

$$\text{DEP } (\mu g) = [(\text{MEP } /194.18) * 222.24]/0.84 \quad [2]$$

Background corrections, hood air corrections, and normalizations

For each metabolite, we summed the total amount excreted in urine samples collected from the time the participants entered the chamber until the last urine sample was collected about 54 hours later, making no adjustments. Using these values, the total doses of DnBP and DEP were calculated from equations 1 and 2. The calculated total doses represent both the exposures that occurred during the 6 h in the chamber as well as those which occurred outside the chamber from 6 to ~ 54 h. Although participants were on a restricted diet, which limited ingestion of DEP and DnBP, they still inhaled and dermally absorbed "background" DEP and DnBP from the time they left the chamber at t = 6 h until their final urine samples were collected at t ~ 54 h. For each participant, we can roughly estimate the dose that occurred in this 48 h period outside the

chamber by using the urine concentrations of metabolites measured in their urine just before entering the chamber coupled with the average volume of urine excreted by each participant in the 48 h period after leaving the chamber. This gives us an estimate of DEP and DnBP uptake over the 48 h after leaving the chamber. We subtract this estimate of "background dose" from the calculated total dose to obtain an estimate of dose during the 6 h in the chamber. Finally, for the exposures when the participants were wearing hoods, we subtract the amount of DEP and DnBP inhaled from hood air (170 and 24 μg respectively). The resulting values represent the dose during exposure in the chamber, corrected for background dose during the period from 6 to ~54 h and also corrected for hood air inhalation intake for the chamber exposures that occurred while participants were wearing hoods. For each participant, the values of the parameters used in these calculations, as well as the final results, are listed in Supplemental Material, Table S4. Finally, for a given six-hour exposure, total uptakes of DEP and DnBP were normalized first by the chamber air concentrations of DEP and DnBP and then by the participant's body mass. Although the potential is small, there may be uptake misclassification due to imperfect corrections for "background dose", hood air and differences in chamber air concentrations.

Results

Metabolite concentrations over time

For each participant, Figure 1 shows creatinine-normalized concentrations of MEP, MnBP and 3OH-MnBP in urine samples collected from the time a participant entered the chamber until 54 hours later (no corrections have been made to the values). When the participants were exposed to elevated chamber concentrations of DEP and DnBP, either wearing a hood or not wearing a hood, urine concentrations of MEP, MnBP and 3OH-MnBP quickly rose, beginning with the

initial urination that occurred after entering the chamber. The metabolite concentrations typically peaked in the first or second urination after the participants exited their 6-hour chamber exposure, and levels remained elevated with respect to pre-chamber levels for at least the next 40 hours. Metabolite concentrations were smaller when the participants were exposed to chamber air while wearing a hood, but the levels were still substantially larger than levels measured before the participants entered the chamber, indicating significant uptake of DEP and DnBP while participants were wearing a hood.

Net amount of metabolite excreted over time

For each participant, Figure 2 shows the net amount of MEP and MnBP excreted from the time exposure began until 54 hours later (no corrections have been made to the values). As anticipated, there is participant-to-participant variability. However, for all participants there is substantial excretion of MEP and MnBP when they are exposed while wearing hoods (i.e., when exposure from chamber air is primarily via the dermal pathway). The net amount of MEP excreted when the participants were wearing hoods is roughly half that excreted when not wearing hoods; for MnBP, the fraction is somewhat less than half. It is also apparent from these plots that, during the initial 12 hours, the excreted amounts of both MEP and MnBP increased faster when participants were not wearing hoods than when wearing hoods.

Total uptake of DEP and DnBP estimated from urinary metabolite levels

As described in the Methods section, the total uptake of DEP and DnBP can be back-calculated from the concentrations of their metabolites in a participant's urine samples. Figure 3 displays box-whisker plots of the net uptakes (μg) of DEP and DnBP, corrected for background and hood air uptakes, and normalized by their measured air concentrations during each exposure

experiment (μg/m^3, Table 1). Separate box-whisker plots are displayed for exposures without a hood (total), exposures with a hood (dermal) and the differences between these two uptakes (inhalation). The median value for dermal uptake of DEP is slightly more than that for its inhalation intake (4.0 vs. 3.8 μg/(μg/m^3 air)). The median value for dermal uptake of DnBP is about 80% of that for its inhalation intake (3.1 vs. 3.9 μg/(μg/m^3 air)).

Total uptakes normalized by air concentrations and body weight

The final normalization that we have made is to divide the net uptakes in Figure 3 by the body weight of each participant. Supplemental Material, Figure S4 is a box-whisker plot displaying the "μg uptake/kg body weight" per "μg/m^3 air" for exposures without a hood (total), with a hood (dermal) and the difference (inhalation). When the uptakes are normalized in this manner, the median dermal uptake of DEP is about 10% more than its inhalation intake (0.048 versus 0.043 μg uptake/kg body wt per μg/m^3 air), and the dermal uptake of DnBP is 82% of its inhalation intake (0.040 versus 0.049 μg uptake/kg body wt per μg/m^3 air).

Differences with age

In Figure 4 the normalized dermal and inhalation uptakes from air ("μg uptake/kg body weight" per "μg/m^3 air") are plotted against the age of the male participants. The older the participant, the larger are the dermal uptakes of both DEP and DnBP from air. Based on this limited sample of six, the impact of age is surprisingly strong. The uptake of DEP by the 66 year-old is five times larger than that of the 27 year-old, while the uptake of DnBP is seven times larger. The inhalation pathway also shows increasing uptake with increasing age for DnBP, but the trend is less pronounced for DEP. In the case of the dermal uptake, Spearman's correlation coefficients between uptake and age are $\rho = 0.9856$ ($p = 0.0003$) and $\rho = 1.0$ ($p = 0.0000$) for DEP and

DnBP, respectively; in the case of the inhalation uptake, the correlation coefficients are ρ = 0.0286 (p = 0.9572) and ρ = 1.0 (p = 0.0000) for DEP and DnBP, respectively. Although their power is weak, these correlation coefficients support expectations based on visual inspection of Figure 4. They indicate that the trend for increased dermal uptake with increasing age is significant for DEP and DnBP, while the trend for increased inhalation uptake is significant for DnBP, but not DEP.

Discussion

Comparison of uptake via dermal pathway with intake via inhalation pathway

Even while wearing a hood, the urinary concentrations of MEP, MnBP and 3OH-MnBP (Figure 1) and the net amount of these metabolites excreted over time (Figure 2) increased significantly as a consequence of a six-hour exposure to elevated air concentrations of DEP and DnBP. Levels and amounts increased much more than could be explained by DEP and DnBP in hood air (Table S4). Additionally, while wearing hoods the total uptakes of DEP and DnBP estimated from urinary metabolite levels – corrected for background and hood air uptakes and normalized by air concentrations (Figure 3) as well as both air concentrations and body weight (Figure S4) – indicate substantial dermal uptake of DEP and DnBP directly from chamber air.

Volume of air inhaled during 6-hour exposure

In Figure 3, where the uptake amounts (μg) have been normalized by the measured air concentrations (μg/m^3), the resulting units are "m^3". For inhalation intakes, this value should correspond to the cubic meters of air inhaled during the six hour exposure in the chamber. The median values shown in the figure for inhalation intakes (3.8 m^3 for DEP, 3.9 m^3 for DnBP) are

remarkably close to the value predicted for an adult male at rest and breathing at a rate of 0.7 m^3/h (US EPA 2011) during a six hour period (4.2 m^3).

Comparison with predicted absorption from air

Although these experiments indicate substantial dermal uptake directly from air for both DEP and DnBP, the measured values for the contribution of the dermal pathway directly from air are less than those predicted in recent papers (Weschler and Nazaroff 2012; 2014; Gong et al. 2014a). The ratio of dermal uptake to inhalation intake predicted in the papers by Weschler and Nazaroff are based on a steady-state model. However, dynamic modeling (Gong et al. 2014a) indicates that more than 48 hours would be required to reach steady-state in the instance of DEP and DnBP; the participants were exposed to elevated concentrations in the chamber for only 6 hours. At the time the participants left the chamber, a large fraction of the DEP and DnBP absorbed by the skin (> 97%) is predicted to be still in the skin. In addition to diffusing into the dermal capillaries, the DEP and DnBP in the skin can desorb to air and the clothing the participants put on after exiting the chamber. Bathing is another sink. The participants were asked not to shower for at least 24 hours after leaving the chamber, but this did not preclude hand washing. Desorption from skin to air and clothing, from the time the participants left the chamber until the time the last urine sample was collected, coupled with washing, would meaningfully reduce the dermal uptake of DEP and DnBP. Gong et al. (2014a) did not include these sinks in their transient model, and so the model over-predicts the amount of DEP and DnBP that reaches the blood for the period from when participants leave the chamber until their last urine sample.

Dermal absorption occurred both on the exposure days that the participants wore hoods and the days without hoods. The lag between dermal absorption and uptake into the blood is apparent from the fact that, although the urine concentrations of MEP, MnBP and 3OH-MnBP peaked shortly after the participants left the chamber, the urine concentrations were still two or more times larger than background 40 hours after leaving the chamber. Such a lag between exposure and excretion was not observed when a participant ingested a known amount of labeled DnBP (Koch et al. 2012), nor would it be anticipated for inhalation of DEP or DnBP.

Under typical indoor conditions occupants tend to be much closer to steady-state, since they are continuously exposed to DEP and DnBP in home and work environments. Note that the chamber concentrations were roughly two orders of magnitude higher than typical indoor levels so that we could distinguish chamber exposures from daily life exposures (e.g., Fromme et al. 2004; Rudel et al. 2003; Wensing et al. 2005; Weschler and Nazaroff 2008). On the other hand, clean clothing may retard dermal uptake from air and bathing may remove DEP and DnBP temporarily sorbed in the stratum corneum. In other words, although people may be closer to steady-state conditions in daily life, resulting in larger ratios of dermal-to-inhalation uptakes than measured in the present study, other exposure variables may have an opposing influence.

Differences with age

The finding that for DEP and DnBP dermal uptake was larger for older participants than younger participants (Figure 4) was unanticipated. The skin's barrier function is influenced by both its thickness and its lipid content. Although the number of epidermal skin cell layers is similar in older and younger humans, older skin has a thinner epidermis (i.e., it is more compact) and has less lipids than younger skin (Harvall and Maibach 1994; WHO 2006). The net effect of these

changes on dermal absorption remains unclear. Roskos et al. (1989) applied various chemicals, radiolabeled with 14C, to the inside surface of the forearms of younger (22-40 yrs) and older (> 65 yrs) human participants. Absorption was assessed using standard radiotracer methodology on urine samples collected for a week following application. Dermal penetration of four hydrophilic compounds (hydrocortisone, benzoic acid, acetylsalicylic acid and caffeine) was significantly less in the older participants than in the younger participants, while dermal penetration of two lipophilic compounds (testosterone and estradiol) was not statistically different between the groups. Both DEP and DnBP are lipophilic compounds. In a review of dermal absorption in aged skin, Harvall and Maibach (1994) conclude that opinions regarding differences in percutaneous absorption between young and old skin "are far from a consensus" and experiments addressing this issue remain non-definitive. This is also the position summarized in a WHO (2006) Environmental Health Criteria document on dermal absorption. We found no studies in the peer reviewed literature that have examined dermal uptake directly from air as a function of age for organic vapors. We recognize that the strong relationship shown in Figure 4 may be due to random error or uncontrolled sources of bias, and we recommend further studies addressing this issue since it has implications for risk assessments in older populations.

Metabolism of phthalates in skin

Using viable skin samples and diffusion cells, Beydon et al. (2010) measured dermal permeation and metabolism of DnBP in samples from humans and other animals. They found that carboxylesterases in skin hydrolyzed DnBP to MnBP in all of the species studied, and that inhibition of carboxylesterase activity retarded passage of DnBP through the skin. In the case of DEHP, a high molecular weight phthalate, Hopf et al. (2014) have shown that it is metabolized

to the monoester MEHP in viable human skin and that it is MEHP, rather than the parent phthalate, that passes into receptor fluid in a diffusion cell. Modeling indicates that for DEHP, the viable epidermis presents greater resistance to transport than does the stratum corneum; conversely for DEP and DnBP, resistance across the stratum corneum is greater than across the viable epidermis (Weschler and Nazaroff 2012). This is significant because if metabolism occurs in the viable epidermis but not the stratum corneum, it will have a larger impact on the overall rate of dermal uptake for DEHP than for DEP and DnBP. Hopf et al. (2014) assume that DEHP first permeates the stratum corneum and is then hydrolyzed, arguing that cutaneous esterases are generally not present in the stratum corneum. Beydon et al. (2010) measured carboxylesterase activity in the epidermis and found that it was only 4.2% of that in whole skin. Although this finding does not distinguish between metabolism in the stratum corneum and viable epidermis, it does indicate greater metabolism after DnBP has passed through the epidermis (i.e., stratum corneum and viable epidermis). If, indeed, there is very little metabolic activity in the stratum corneum, then in terms of risk assessment it probably does not matter a great deal whether hydrolysis of DEP and DnBP occurs before or after the phthalate enters the blood. However, such a conclusion requires that the relative dermal toxicity of the monoester is comparable to that of the parent compound.

Impact of clothing

In this study the human participants wore only shorts; the rest of their skin was directly exposed to air. A preliminary evaluation of the impact of clothing on these exposures was conducted concurrently with this study and will be described in a future publication.

Other phthalates

For dermal uptake directly from air to contribute significantly to total body burden, an organic compound must possess the right physical-chemical properties – a relatively large value for its skin lipid/air partition coefficient (K_{sc_g}) and a relatively small molecular weight (Weschler and Nazaroff, 2012; 2014; Gong et al. 2014a). Although most phthalate esters have values for K_{sc_g} that are favorable for dermal absorption from air, higher molecular weight phthalates such as butyl benzylphthalate (BBzP), di(2-ethylhexyl) phthalate (DEHP) and di(isononyl) phthalate (DiNP) tend to have low gas-phase concentrations. This results in kinetic constraints on the flux from air-to-skin; it is too small for dermal uptake from air to be an important pathway for compounds such as DEHP and DiNP. Although DEHP has been measured at high levels in skin wipes (Gong et al. 2014b; Kim H-H et al. 2011), this likely reflects transfer to skin via contact with DEHP contaminated surfaces. In summary, dermal absorption directly from indoor air should be included as a contributory exposure pathway in risk assessments of low molecular weight phthalates, but this pathway is anticipated to be of decreasing importance with increasing molecular weight.

Conclusions

This study has demonstrated that for human participants, following a six-hour "dermal only" exposure to elevated gas-phase concentrations of DEP and DnBP, the levels of the metabolites MEP, MnBP and 3OH-MnBP in urine samples collected over the next two days were roughly half those measured in urine samples following a six-hour "dermal + inhalation" exposure. Although earlier assessments of human exposure to phthalate esters have included the dermal pathway (e.g., Guo and Kannan 2011; Koch et al. 2013; Wittassek et al. 2011; Wormuth et al.

2006), it is only recently that dermal absorption directly from air has been part of such assessments (Bekö et al. 2013; Gaspar et al. 2014). The present study provides support for including this pathway in risk assessments. However, the ratios of dermal uptake to inhalation intake for DEP and DnBP assumed in the modeling studies of Bekö et al. and Gaspar et al. are larger than those measured in the current study. This apparent disparity may reflect the fact that in the current study the participants were exposed to elevated levels of DEP and DnBP for only 6 hours, whereas dynamic modeling indicates that more than 40 hours would be required to reach steady-state and maximal uptake via the dermal pathway in comparison to the inhalation pathway.

References

Bader M, Wrbitzky R, Blaszkewicz M, Schaper M, van Thriel C. 2008. Human volunteer study on the inhalational and dermal absorption of n-methyl-2-pyrrolidone (nmp) from the vapour phase. Archives of Toxicology 82:13-20.

Bekö G, Weschler CJ, Langer S, Callesen M, Toftum J, Clausen G. 2013. Children's phthalate intakes and resultant cumulative exposures estimated from urine compared with estimates from dust ingestion, inhalation and dermal absorption in their homes and daycare centers. PLoS One 8:e62442.

Beydon D, Payan JP, Grandclaude MC. 2010. Comparison of percutaneous absorption and metabolism of di-n-butylphthalate in various species. Toxicology in vitro : an international journal published in association with BIBRA 24:71-78.

Bolgar M., Hubball, J., Groeger, J., meronek, S. (2008) Handbook for the Chemical Analysis of Plastic and Polymer Additives. CRC Press, Taylor & Francis group, Boca Raton.

CDC (Centers for Disease Control and Prevention). 2015. Fourth National Report on Human Exposure to Environmental Chemicals, Updated Tables, February 2015. Atlanta, GA: Centers for Disease Control and Prevention. Available: http://www.cdc.gov/biomonitoring/pdf/FourthReport_UpdatedTables_Feb2015.pdf [accessed 22 March 2015].

DIN ISO 16000-6 (2012). Indoor air - Part 6: Determination of volatile organic compounds in indoor and test chamber air by active sampling on Tenax TA sorbent, thermal desorption and gas chromatography using MS/FID. Beuth Verlag, Berlin.

Dodson RE, Nishioka M, Standley LJ, Perovich LJ, Brody JG, Rudel RA. 2012. Endocrine disruptors and asthma-associated chemicals in consumer products. Environ Health Perspect 120:935-943.

Einax J.W., Zwanzinger H.W., Geiß S. (1997) Chemometrics in Environmental Analysis. WILEY-VCH, Weinheim.

Ferguson KK, Loch-Caruso R, Meeker JD. 2011. Urinary phthalate metabolites in relation to biomarkers of inflammation and oxidative stress: NHANES 1999-2006. Environ Res 111:718-726.

Fromme H, Lahrz T, Piloty M, Gebhart H, Oddoy A, Ruden H, 2004. Occurrence of phthalates and musk fragrances in indoor air and dust from apartments and kindergartens in Berlin (Germany). Indoor Air 14: 188-195.

Gaspar FW, Castorina R, Maddalena RL, Nishioka MG, McKone TE, Bradman A. 2014. Phthalate exposure and risk assessment in California child care facilities. Environ Sci Technol 48:7593-7601.

Gong M, Zhang Y, Weschler CJ. 2014a. Predicting dermal absorption of gas-phase chemicals: Transient model development, evaluation, and application. Indoor Air 24:292-306.

Gong M, Zhang Y, Weschler CJ. 2014b. Measurement of phthalates in skin wipes: Estimating exposure from dermal absorption. Environ Sci Technol. 48:7428-7435.

Guo Y, Kannan K. 2011. Comparative assessment of human exposure to phthalate esters from house dust in China and the United States. Environ Sci Technol 45:3788-3794.

Harvell JD, Maibach HI. 1994. Percutaneous-absorption and inflammation in aged skin - a review. Journal of the American Academy of Dermatology 31(6):1015-1021.

Hatch EE, Nelson JW, Stahlhut RW, Webster TF. 2010. Association of endocrine disruptors and obesity: Perspectives from epidemiological studies. Int J Androl 33:324-332.

Hopf NB, Berthet A, Vernez D, Langard E, Spring P, Gaudin R. 2014. Skin permeation and metabolism of di(2-ethylhexyl) phthalate (DEHP). Toxicol Lett 224:47-53.

Jaakkola JJ, Knight TL. 2008. The role of exposure to phthalates from polyvinyl chloride products in the development of asthma and allergies: A systematic review and meta-analysis. Environ Health Perspect 116:845-853.

Kim H-H, Yang J-Y, Kim S-D, Yang S-H, Lee C-S, Shin D-C, et al. 2011. Health risks assessment in children for phthalate exposure associated with childcare facilities and indoor playgrounds. Environ Health Toxicol 26:e2011008.

Kim Y, Ha EH, Kim EJ, Park H, Ha M, Kim JH, et al. 2011. Prenatal exposure to phthalates and infant development at 6 months: Prospective mothers and children's environmental health (MOCEH) study. Environ Health Perspect 119:1495-1500.

Kissel JC. 2011. The mismeasure of dermal absorption. J Expo Sci Environ Epidemiol 21:302-309.

Koch HM, Gonzalez-Reche LM, Angerer J. 2003. On-line clean-up by multidimensional liquid chromatography-electrospray ionization tandem mass spectrometry for high throughput quantification of primary and secondary phthalate metabolites in human urine. J Chromatogr B Analyt Technol Biomed Life Sci 784:169-182.

Koch HM, Becker K, Wittassek M, Seiwert M, Angerer J, Kolossa-Gehring M. 2007. Di-n-butylphthalate and butylbenzylphthalate - urinary metabolite levels and estimated daily intakes: Pilot study for the German environmental survey on children. J Expo Sci Environ Epidemiol 17:378-387.

Koch HM, Wittassek M, Brüning T, Angerer J, Heudorf, U. 2011. Exposure to phthalates in 5–6 years old primary school starters in Germany – a human biomonitoring study and a cumulative risk assessment. Int J Hyg Environ Health 214:188–195.

Koch HM, Christensen KL, Harth V, Lorber M, Bruning T. 2012. Di-n-butyl phthalate (DnBP) and diisobutyl phthalate (DiBP) metabolism in a human volunteer after single oral doses. Arch Toxicol 86:1829-1839.

Koch HM, Lorber M, Christensen KL, Palmke C, Koslitz S, Bruning T. 2013. Identifying sources of phthalate exposure with human biomonitoring: Results of a 48h fasting study with urine collection and personal activity patterns. Int J Hyg Environ Health 216:672–681.

Langer S, Beko G, Weschler CJ, Brive LM, Toftum J, Callesen M, et al. 2014. Phthalate metabolites in urine samples from Danish children and correlations with phthalates in dust samples from their homes and daycare centers. Int J Hyg Environ Health 217(1):78-87.

Piotrowski J. 1967. Further investigations on the evaluation of exposure to nitrobenzene. Br J Ind Med 24:60-65.

Piotrowski JK. 1971. Evaluation of exposure to phenol: Absorption of phenol vapour in the lungs and through the skin and excretion of phenol in urine. Br J Ind Med 28:172-178.

Preuss R, Koch HM, Angerer J. 2005. Biological monitoring of the five major metabolites of di-(2-ethylhexyl)phthalate (DEHP) in human urine using column-switching liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 816:269-280.

Roskos KV, Maibach HI, Guy RH. 1989. The effect of aging on percutaneous absorption in man. J Pharmacokinet Biopharm 17(6): 617-630.

Rudel RA, Camann DE, Spengler JD, Korn LR, Brody JG. 2003. Phthalates, alkylphenols, pesticides, polybrominated diphenyl ethers, and other endocrine-disrupting compounds in indoor air and dust. Environ Sci Technol 37: 4543-4553.

Schindler BK, Esteban M, Koch HM, Castano A, Koslitz S, Canas A, et al. 2014. The European COPHES/DEMOCOPHES project: towards transnational comparability and reliability of human biomonitoring results. Int J Hyg Environ Health 217(6): 653-661.

Schripp T., Salthammer T, Fauck C., Bekö G., Weschler C. J. 2014. Latex paint as a delivery vehicle for diethylphthalate and di-n-butylphthalate: predictable boundary layer concentrations and emission rates, Sci Total Environ, 494-495: 299-305.

Svensson K, Hernandez-Ramirez RU, Burguete-Garcia A, Cebrian ME, Calafat AM, Needham LL, et al. 2011. Phthalate exposure associated with self-reported diabetes among Mexican women. Environ Res 111:792-796.

Swan SH, Main KM, Liu F, Stewart SL, Kruse RL, Calafat AM, et al. 2005. Decrease in anogenital distance among male infants with prenatal phthalate exposure. Environ Health Perspect 113:1056-1061.

Taussky HH. 1954. A microcolorimetric determination of creatine in urine by the Jaffe reaction. J Biol Chem 208: 853-861.

U.S. EPA. Exposure Factors Handbook 2011 Edition (Final). U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-09/052F, 2011. Available: http://www.epa.gov/ncea/efh/pdfs/efh-complete.pdf [accessed 22 March 2015].

Wensing M, Uhde E, Salthammer T. 2005. Plastics additives in the indoor environment--flame retardants and plasticizers. Sci Total Environ 339: 19-40.

Weschler CJ, Nazaroff WW. 2008. Semivolatile organic compounds in indoor environments. Atmos Environ 42: 9018-9040.

Weschler CJ, Nazaroff WW. 2012. SVOC exposure indoors: Fresh look at dermal pathways. Indoor Air 22:356-377.

Weschler CJ, Nazaroff WW. 2014. Dermal uptake of organic vapors commonly found in indoor air. Environ Sci Technol 48:1230-1237.

WHO (2006). Dermal Absorption. Environmental Health Criteria (EHC) 235. ISBN 978 92 4 157235 4, Geneva, Switzerland. Available: http://www.who.int/ipcs/publications/ehc/ehc235.pdf?ua=1 [accessed 22 March 2015].

Whyatt RM, Liu X, Rauh VA, Calafat AM, Just AC, Hoepner L, et al. 2012. Maternal prenatal urinary phthalate metabolite concentrations and child mental, psychomotor, and behavioral development at 3 years of age. Environ Health Perspect 120:290-295.

Wittassek M, Koch HM, Angerer J, Bruening T. 2011. Assessing exposure to phthalates - the human biomonitoring approach. Molecular Nutrition & Food Research 55:7-31.

Wormuth M, Scheringer M, Vollenweider M, Hungerbuhler K. 2006. What are the sources of exposure to eight frequently used phthalic acid esters in europeans? Risk Analysis 26:803-824.

Wypych, G. 2012. Handbook of Plasticizers, 2nd ed. Toronto: ChemTec Publishing.

Zota AR, Calafat AM, Woodruff TJ. 2014. Temporal trends in phthalate exposures: Findings from the national health and nutrition examination survey, 2001-2010. Environ Health Perspect 122:235-241.

Table 1. Average concentrations (minimum-maximum) of DEP and DnBP measured in the chamber during the six-hour exposure intervals.

Day of Exposure	**Group**	**Status**	**DEP (μg/m^3)**	**DnBP (μg/m^3)**
Tuesday, 1st week	Group 1	Hoods	250 (240-280)	123 (120-132)
Wednesday, 1st week	Group 2	No hoods	233 (225-252)	114 (108-122)
Tuesday, 2nd week	Group 1	No hoods	291 (282-317)	140 (135-149)
Wednesday, 2nd week	Group 2	Hoods	284 (240-353)	140 (122-163)

Figure Legends

Figure 1. Urine concentrations (creatinine-normalized) of MEP, MnBP and 3OH-MnBP in participants' urine samples collected from shortly before entering the chamber until 54 hours after the six-hour exposure began. No adjustments have been made to the values other than normalization by creatinine.

Figure 2. Net amount of MEP and MnBP excreted by each of the six participants from the time exposure began until 54 hours later. No adjustments have been made to the values.

Figure 3. Box-whisker plots displaying the uptakes (μg) of DEP and DnBP, corrected for uptakes occurring outside the chamber and from hood air, normalized by measured chamber air concentrations during each exposure experiment (μg/m^3, Table 1), for exposures without a hood (total), exposures with a hood (dermal) and the differences between these two uptakes (inhalation). Boxes extend from the 25th to the 75th percentile, horizontal bars represent the median, and whiskers indicate the 10th and 90th percentiles. The markers indicate individual results for each of the six participants.

Figure 4. Dermal and inhalation uptakes from chamber air, corrected for uptakes occurring outside the chamber and from hood air, and normalized by chamber air concentrations and body weight ("μg uptake/kg body weight" per "μg/m^3 air") plotted against the age of the male participants.

Figure 1.

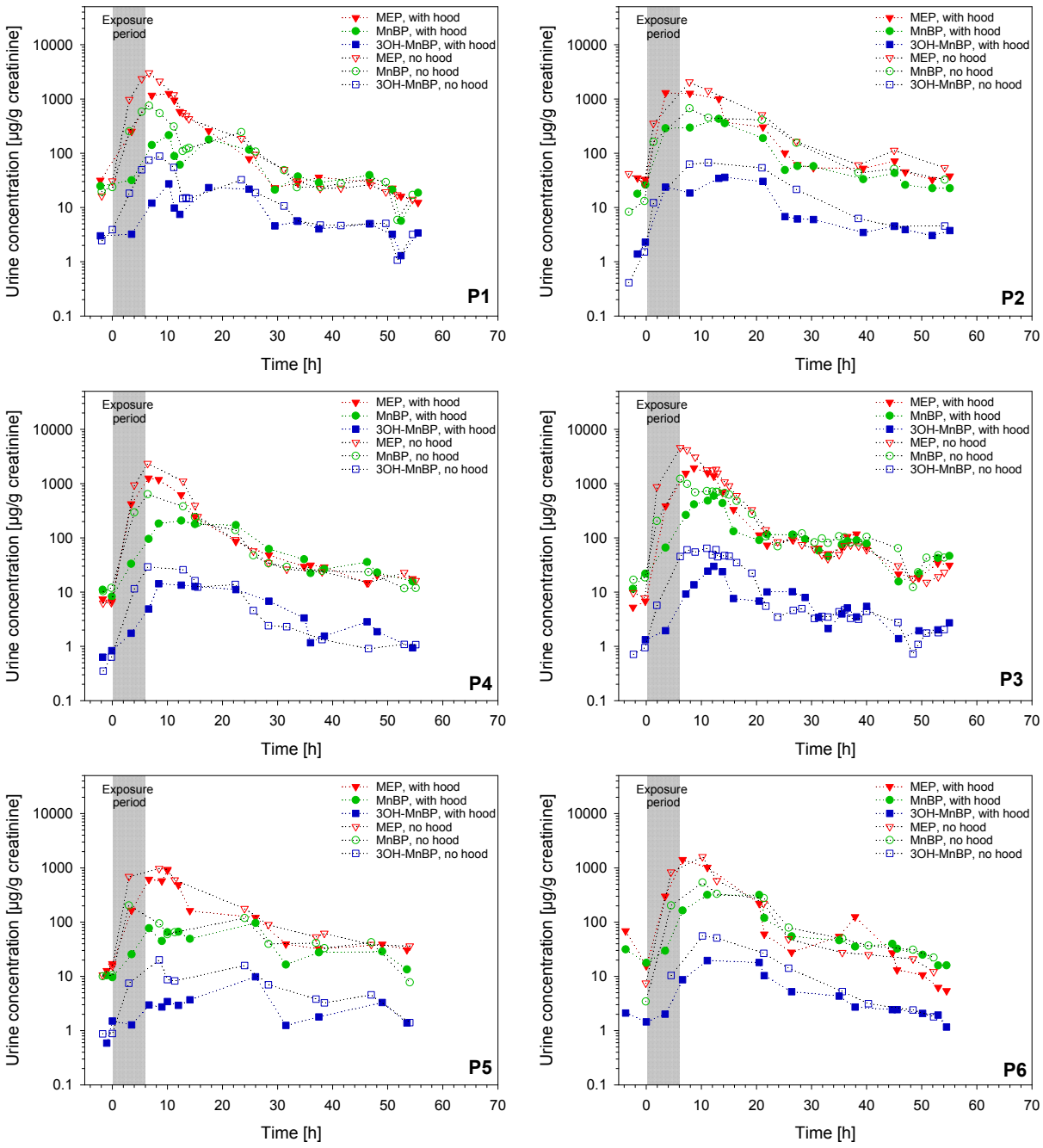

Exposure period
MEP, with hood
MnBP, with hood
3OH-MnBP, with hood
MEP, no hood
MnBP, no hood
3OH-MnBP, no hood
Urine concentration [µg/g creatinine]
Time [h]
P1
P2
P3
P4
P5
P6

Figure 2.

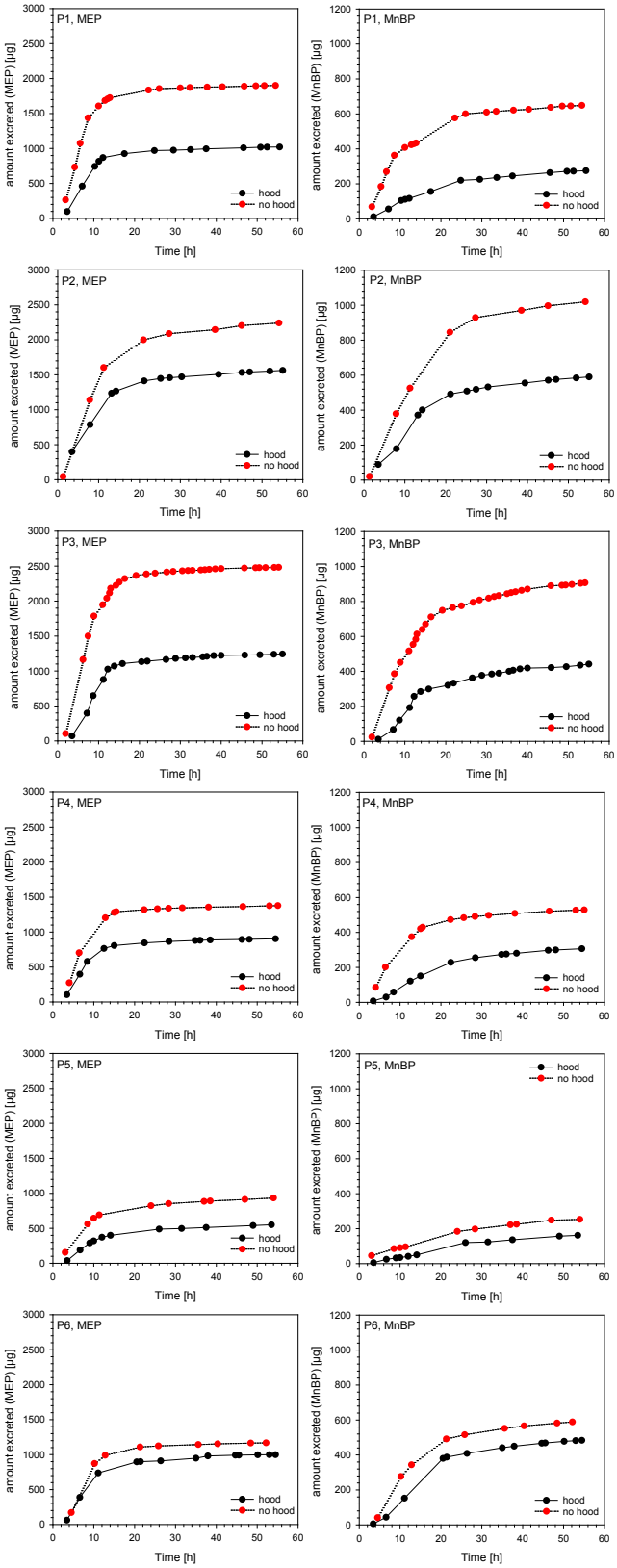

P1, MEP
P1, MnBP
P2, MEP
P2, MnBP
P3, MEP
P3, MnBP
P4, MEP
P4, MnBP
P5, MEP
P5, MnBP
P6, MEP
P6, MnBP
amount excreted (MEP) [µg]
amount excreted (MnBP) [µg]
Time [h]
hood
no hood

Figure 3.

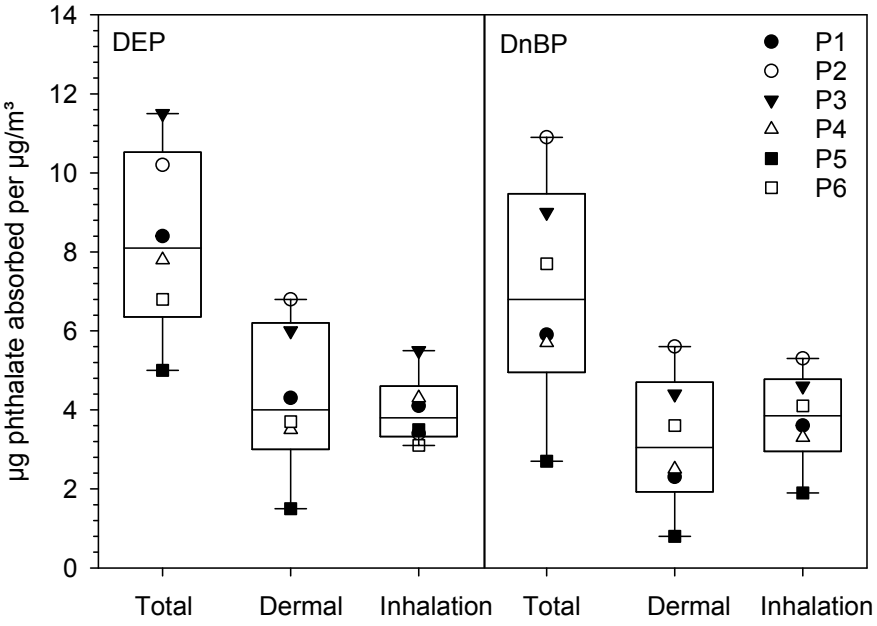
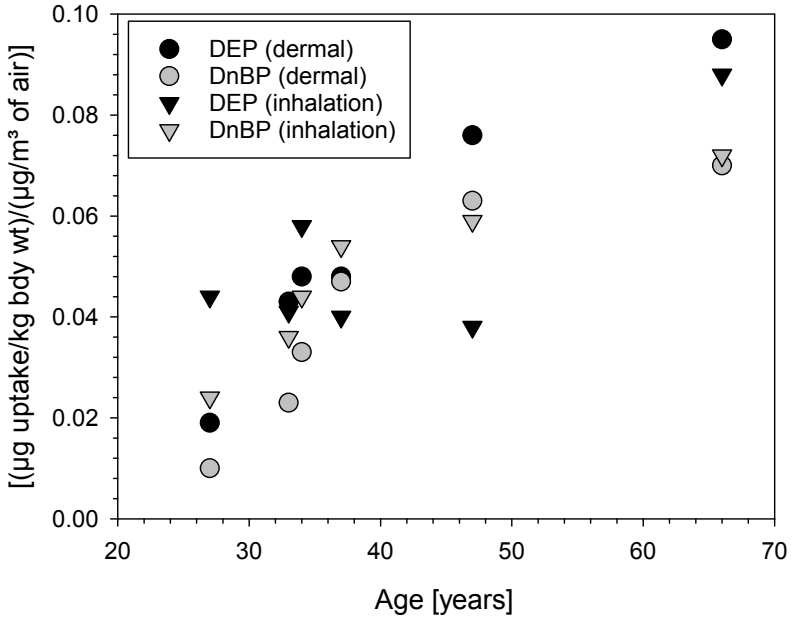

DEP
DnBP
P1
P2
P3
P4
P5
P6
µg phthalate absorbed per µg/m³
0
2
4
6
8
10
12
14
Total
Dermal
Inhalation
Total
Dermal
Inhalation

Figure 4.


DEP (dermal)
DnBP (dermal)
DEP (inhalation)
DnBP (inhalation)
[(µg uptake/kg bdy wt)/(µg/m³ of air)]
0.00
0.02
0.04
0.06
0.08
0.10
20
30
40
50
60
70
Age [years]